PATENT SPECIFICATION

809,912

Date of Application and filing Complete Specification Sept. 7, 1955.
No. 25680/55
Application made in United States of America on Sept. 7, 1954.
Application made in United States of America on Nov. 10, 1954.
Application made in United States of America on Jan. 12, 1955.
Application made in United States of America on Feb. 11, 1955
Complete Specification Published March 4, 1959.

Index at acceptance: —Classes 2(3), C3A7(A4: B: C: F2: G1: G2: H); 41, B(2C: 18); and 81(1), B2(B3: D: E: G: H: J: L: N: P: Q: R: S).

International Classification: —A61k. B01d. C07g.

COMPLETE SPECIFICATION

Active Substance from Plants of the *Rauwolfia* Species and process for Manufacturing same

We, CIBA LIMITED, a body corporate organised according to the laws of Switzerland, of Basle, Switzerland, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention provides a new pure compound having valuable pharmacological activity, which is hereinafter called deserpidine, and salts thereof, and to a process for the manufacture thereof.

According to the present invention deserpidine is isolated from the mother liquors from a reserpine crystallisation obtainable in a process for the isolation of reserpine from plants of the *Rauwolfia* species, more particularly from *Rauwolfia canescens*, or furthermore from *Rauwolfia hirsuta* (also known as *Rauwolfia heterophylla*), *Rauwolfia tetraphylla*, *Rauwolfia indecora*, *Rauwolfia vomitoria Afz.* and *Rauwolfia cubana*, preferably from the roots of these plants.

Deserpidine melts at 228—232° C. and possesses an optical rotation $[\alpha]_D^{24.5} = -137 \pm 1°$ (in chloroform). Analysis shows the following results: C=66.42; H=6.76; N=4.89; OCH_3=26.90. Its empirical formula appears to be $C_{32}H_{38}O_8N_2$.

Deserpidine, in its behaviour towards solvents and acids, shows a strong similarity to reserpine. It is fairly soluble in acetone, methanol, benzene, ethyl acetate, dilute acetic acid and halogenated aliphatic hydrocarbons, such as methylene chloride, ethylene chloride, trichloro ethylene and chloroform, and difficultly soluble or insoluble in ether, petrol ether, hexane and water. The new compound can be crystallized, for example, from methanol, acetone or ethyl acetate. Crystallized from methanol, it forms colourless prismatic needles (α-form) of the above melting point. It can also be obtained from methanol in the form of needles melting at 230—232° (β-form) and in the form of prisms having double melting point of 138° and 226—232° with resolidifying at 175° (γ-form). It is a weak base [pKa^1 6.68 (40% methanol)] and forms salts such as the difficultly soluble hydrochloride, a nitrate, sulfate, oxalate or a picrate. It dissolves in an excess of dilute acetic acid, thus forming the acetate. Its ultraviolet spectrum in ethanol has a maximum at 272 mu (log E= approx. 4,2); another maximum at 217 mu (log E=approx. 4,8) and a minimum at 244 mu (log E=approx. 3,9). The infrared absorption spectrum of the α-form [in Nujol (Registered Trade Mark)] shows the following characteristic bands classified in groups of diminishing strength; strong bands at 1731, 1715, 1590, 1504, 1415, 1332, 1274, 1250, 1226, 1124, 1100, 1005, 977, 761, 728 cm^{-1}; medium bands at 1357, 1349, 1190, 1174, 1108, 1100 cm^{-1}; medium weak bands at 3246, 1065, 1043, 1030, 1018, 870, 770, 737 cm^{-1}; weak bands at 942, 927, 915, 901, 854, 835, 799 cm^{-1}.

Deserpidine has a complex pharmacological action, which is primarily characterized by a strong, relatively fast onsetting, and prolonged sedative effect, and a less pronounced hypotensive effect. It can be used as medicament for producing sedation and for the treatment of hypertension. For therapeutical use deserpidine or its salts may be made up into pharmaceutical compositions which comprise deserpidine or its salts substantially free from combined alkaloids and other materials associated with plants of the *Rauwolfia* species together with a pharmaceutical adjuvant as a carrier. The compositions provided by the invention may be in any suitable solid or liquid dosage form, containing about 0.05—100 mg, preferably 0.1—20 mg. of active substance, especially in a form suitable for oral or parenteral administration e.g. tablets, powder, capsules, pills, solutions, emulsions or suspensions, e.g. in the form of ampouled injectable

[Pric

solutions. As pharmaceutical carriers there may be employed materials or mixtures of such which do not react with deserpidine and are therapeutically useful. Substances or mixtures thereof, such as water, gelatine, lactose, starch, magnesium stearate, talc, vegetable oils, benzyl alcohol, ascorbic acid, gums, glycols such as propylene glycol or polyalkylene glycol, petroleum jelly, cholesterol, tragacanth, alcohol or others may be employed. In preparing the novel compositions the pure deserpidine or its salts are admixed with the pharmaceutical carrier and formulated in the desired dosage unit form according to pharmaceutical practice. The compositions may be sterilized and may contain auxiliary substances such as preservative, stabilizing, wetting or emulsifying substances, salts for the control of the osmotic pressure or buffer substances or besides pure deserpidine other therapeutically active substances, for example such as are used in combination with reserpine. As therapeutically active substances which may be present in the compositions, there may be mentioned other hypotensive active substances, such as ganglionic blockers, e.g. N:N:N¹:N¹-3-pentamethyl - N:N¹ - diethyl - 3 - azapentylene-1:5-diammonium dibromide, hexamethylene bis - trimethyl - ammonium bromide, pentamethylene bis-methylpyrrolidinium ditartrate or 2-(2¹-dimethylaminoethyl)-4:5:6:7-tetrachloroisoindoline dimethochloride; adrenergic blockers, e.g. 2-(N¹-*p*-toyl-N¹-*m*-hydroxyphenyl-aminomethyl)-imidazoline or active derivatives of ergot alkaloids; hydrazinopyridazines e.g. 1,4-dihydrazino-phthalazine or 1-hydrazino-phthalazine; *Rauwolfia* alkaloids in pure form e.g. reserpine; analogues of reserpine e.g. 3,4,5-trimethoxy-cinnamoyl methyl reserpate or acetyl-methyl reserpate; analogues of deserpidine, e.g. 3,4,5-trimethoxy-cinnamoyl methyl deserpidate as described, for example, in Specification No. 32094/55 (Serial No. 809,913). There may also be mentioned sedative active substances such as barbiturates, 3-ethyl-3-phenyl-2,6-dioxo-piperidine, N-(3¹-dimethylaminopropyl) - 3 - chloro - phenthiazine; central nervous stimulants, such as methyl α-piperidyl-(2)-phenyl acetate or *dl*-α-methylphenylethylamine; cholinergic blocking agents such as diethylamino-ethyl α-cyclohexyl-α-phenyl-α-hydroxy acetate methobromide, atropine or diethylaminoethyl 9-xanthenecarboxylate methobromide; or anti-histaminics such as 2-[benzyl-(2¹-dimethyl-aminoethyl)-amino]-pyridine.

In contrast to the heterogeneous and crude preparations from plants of the *Rauwolfia* species, the present invention provides many advantages with compositions made up from pure deserpidine obtained according to the invention. The exact amount of the dose to produce a certain effect can be indicated. In addition, the effect and potency of such compositions are uniform and unvarying. Deserpidine, being a pure crystalline alkaloid, lends itself to the preparation of novel solutions which can be administered parenterally, for example, intravenously, which is not possible with the whole roots and crude extracts.

Deserpidine is also a valuable intermediate product for the preparation of other pharmacologically active and therapeutically useful compounds. Thus, deserpidine can be converted by partial hydrolysis into methyl deserpidate, from which new valuable esters can be obtained, as described for example, in Specification No. 32094/55. Such esters are for example O-(3:4-dimethoxy-benzoyl)-methyl deserpidate and O-furoyl-(2)-methyl deserpidate, which show sedative and hypotensive activity and can be used as medicaments causing sedation and for the treatment of hypertension.

The process for the preparation of the new active compound consists in isolating deserpidine from the mother liquors from a reserpine crystallisation obtainable in a process for the isolation of reserpine from plants of the *Rauwolfia* species, the isolation being carried out with use of at least one of the following methods:—

(a) Treating the mother liquors with acids or salts suitable for salt formation with weak bases and separating the salts obtained;

(b) Decomposing the mother liquors; and

(c) Crystallisation.

Thus it is possible to isolate deserpidine from the mother liquors to be used according to the invention by crystallization, preferably using the method of fractional crystallization, from solvents such as methanol, acetone and ethyl acetate. Advantageously before the crystallization the mother liquors are decomposed with an adsorbing agent, such as aluminium oxide, silicic acid, diatomaceous earth or another silicate. The adsorption procedure may be repeated. From the adsorbing agent deserpidine is then eluted preferably by means of benzene. Decomposition may also be performed by electrodialysis or paper-electrophoresis. It is further possible to isolate deserpidine by treating the crude mother liquors with acids or salts suitable for salt formation with weak bases so as to obtain a separation and then isolation of deserpidine from its salts thus obtained.

To obtain the mother liquors to be used as starting material and to perform the isolation of deserpidine in pure form therefrom, the following methods may be used: A preferred procedure for obtaining the new compound is characterized by extracting plant material of plants of the *Rauwolfia* species or a crude extract obtained therefrom in a manner adapted to the properties of the new compound, with an organic solvent only partially miscible with water, especially a weakly polar to non-polar one, so as to produce a sedative active extract, and after having separated any

reserpine present isolating deserpidine therefrom using purifying methods.

As starting material there is used, according to this procedure, for example, the finely ground plant material of *Rauwolfia* plants, especially root material and preferably material from *Rauwolfia canescens*, or also *Rauwolfia hirsuta*, *Rauwolfia tetraphylla*, *Rauwolfia indecora*, *Rauwolfia cubana* and *Rauwolfia vomitoria Afz.* Crude extracts obtained therefrom which are also suitable for the above mentioned extraction with the organic solvent which is only partially miscible with water are advantageously alcohol extracts, such as extracts obtained with the use of lower alkanols, preferably methanol or ethanol, which may have been kneaded with water and then again separated from water, and which have preferably been treated with a lipoid solvent such as petroleum ether or hexane. The so-called "oleoresins" also suitable as crude extracts may be obtained as follows:—

A crude alcohol extract of the finely ground plant material of *Rauwolfia* species is evaporated to dryness and then preferably kneaded first with water; the insoluble residue thus obtained is then treated with 2-N-hydrochloric acid, dried and extracted continuously with a lipoid solvent, such as petroleum ether, the brown residue thereby produced is then treated with 95 per cent ethanol and the brown solution obtained after filtration with suction evaporated to dryness.

For further purification the crude extracts may be extracted with cyclic ethers or acetals, such as tetrahydrofurane, dioxane, or dioxolanes such as glycol acetal, whereby resinous components are removed.

According to the invention, the plant material or the crude extracts obtained therefrom, for example as indicated above, are extracted with an organic solvent only partially miscible with water, especially a weakly to non-polar one. Examples of weakly polar to non-polar solvents are preferably halogenated aliphatic hydrocarbons, such as methylene chloride, ethylene chloride, trichloroethylene or chloroform, or solvents such as benzene, ethyl acetate, ether or mixtures thereof. This extraction may advantageously be carried out in the presence of a polar solvent, such as a lower alkanol, e.g. methanol or ethanol, and/or in the presence of water, which may be added. There may be added to the water, bases, acids or salts such as ammonia, inorganic or organic acids such as hydrochloric acid, phosphoric acid or acetic acid, sodium carbonate, sodium bicarbonate, potassium biphosphate or potassium bisulfate. If a mixture of the organic solvent only partially miscible with water and a polar solvent is used, it is of advantage to separate the solution obtained into two phases by adding water. It is, however, possible to distribute the starting material between a mixture of water and a polar solvent and the organic solvent which is only partially miscible therewith. The distribution should preferably take place over several separation stages. The fractions with the organic solvent only partially miscible with water thus obtained can be evaporated to dryness and the residue worked up, after the separation of any reserpine present, directly to deserpidine according to the invention.

Another method of obtaining a crude extract suitable for carrying out the process consists in treating plant material of *Rauwolfia* plants or a crude extract therefrom obtained, for example as indicated above, with an aqueous acid agent so as to produce a sedative active aqueous acidic solution containing the new sedative and hypotensive active compound. Aqueous acid agents suitable for this purpose are, for example, lower fatty acids such as formic acid, acetic acid or propionic acid, or a phosphoric acid or an acid salt of a polybasic acid. The acid solution of the new compound thus obtained can, for the purpose of further working up, either be concentrated to a smaller volume or diluted with water or left unchanged. It may be treated with lipoid solvents, such as petrol ether or hexane. The thus obtained acid solution is then extracted according to the invention with the organic solvent which is only partially miscible with water. The obtained extracts can be washed neutral and evaporated to dryness.

The above mentioned methods can be combined in an appropriate manner. Thus, plant material of the *Rauwolfia* species may be treated simultaneously with an alcohol, the aqueous acid agent and the organic solvent only partially miscible with water, or the plants may first be extracted with an alcohol, which extract is then dissolved in the aqueous acid agent, such as acetic acid, which, in turn, is then extracted with the organic solvent, preferably after treatment with a lipoid solvent. In the preparation of the extracts useful for the isolation of deserpidine, it is also possible to work in such a way that at any step in the process an extract, for example an extract obtained with methanol from roots, is mixed with a carrier substance such as "Hyflo" (Registered Trade Mark), "Charcel DIC" (diatomaceous earths) or another silicate, and the remaining steps in the process are carried out. The active substance can, for instance, be extracted from the dry carrier substance with one of the mentioned aqueous acid agents or by means of an organic solvent, preferably in admixture with a polar solvent. To facilitate the isolation of deserpidine, the extracts may be further subjected to an electrodialysis or paper-electrophoresis.

Other methods for the preparation of the new compound consist in treating crude extracts obtained in accordance with the invention from plants of the *Rauwolfia* species with acids or salts suitable for salt formation with

weak bases, so as to obtain a separation and then isolating deserpidine from its thus obtained salts which are in admixture with accompanying substances, or in subjecting crude extracts, for example, methanolic extracts, preferably after a precipitation with aqueous ammonia, to an electrodialysis or a paper ionophoresis, for example, in aqueous acetic acid solution, thus removing resinous components and after having separated any reserpine present, isolating deserpidine.

Thus a crude extract can be treated with nitrates, chlorides, sulphates, picrates, picrolonates, perchlorates, or sulphonates, or complex salts of heavy metals suitable for the formation of difficultly soluble alkaloid salts, e.g. phosphotungstates, phosphomolybdates, mercuric iodates or the corresponding acids. Depending on the nature of the salts or acids used, the reaction may be carried out, for example, in a alcoholic such as methanolic solution. An acetic acid solution may be treated with a mixture of sodium nitrate and sodium chloride and calcium oxide and the salt precipitate separated. From the crude salts deserpidine may advantageously be isolated by setting free the bases and isolating the deserpidine therefrom in accordance with the invention. Thus the above mentioned precipitate may be treated with methanolic ammonia and deserpidine isolated therefrom by disintegration on an adsorbent, or by fractional crystallization, or preferably a combination of these methods. These methods may be combined with the above mentioned processes in an appropriate way.

In carrying out the processes of the invention, it is of special importance to know that deserpidine (α-form or in solution) possesses in the IR-spectrum a band at 728 cm^{-1} not shared by reserpine, whereas deserpidine does not show the 1625 cm^{-1} band of reserpine.

Depending on the method of working, deserpidine is obtained in the form of the base or its salts. From the base, therapeutically useful salts can be obtained, such as that of the hydrohalic acids, sulfuric acid, nitric acid, perchloric acid, phosphoric acids, acetic acid, propionic acid, lactic acid, oxalic acid, succinic acid, malic acid, tartaric acid, citric acid, ascorbic acid, methane sulfonic acid, ethane sulfonic acid, hydroxyethane sulfonic acid, benzoic acid, salicylic acid, *p*-amino-salicylic acid and toluene parasulphonic acid, by reacting the deserpidine with such acids in the presence or absence of a diluent. The salts can also be obtained by double decomposition. Of especial interest is deserpidine phosphate for reason of its water solubility. Thus, an aqueous solution for injection can be made by dissolving deserpidine phosphate in water for injection.

The following examples illustrate the invention, the relation between parts by weight and parts by volume being the same as that between the gram and the cubic centimeter:—

EXAMPLE 1.

500 parts by weight of dried, finely ground roots of *Rauwolfia canescens* are extracted batch-wise with methanol at its boiling point, using the following volumes and times, and filtering each extract while hot: 2000 parts by volume, 1 hour; 1000 parts by volume, 45 minutes; 1000 parts by volume, 30 minutes; 1000 parts by volume, 30 minutes. The extracts are combined and evaporated *in vacuo* to 75 parts by volume of a thick syrupy solution. After the addition of 75 parts by volume of methanol and 150 parts by volume of acetic acid of 15 per cent strength with adequate mixing, the solution is extracted with 2 portions each of 100 parts by volume of hexane. The combined hexane extracts are extracted with 15 parts by volume of acetic acid of 15 per cent strength. The latter extract is added to the above acetic acid phase which is then extracted with 3 portions each of 75 parts by volume and 1 portion of 50 parts by volume of ethylene chloride. The first 3 extracts are combined and washed with 60 parts by volume of 2N sodium carbonate solution and then with 60 parts by volume of distilled water. These washing solutions are saved and used for the washing of the 4th and final ethylene chloride extract. The combined ethylene chloride extracts are dried over sodium sulfate, filtered and evaporated *in vacuo* to a constant weight of a tan, frothy solid. 1 part by weight of this residue is dissolved in 1.5 parts by volume of warm methanol and the solution cooled to 5° C. for 18 hours, whereby crystallization of a mixture containing principally reserpine sets in. After filtering this mixture and washing it with cool methanol, the filtrate is freed of solvent *in vacuo*. 2 parts by weight of the resulting red-brown solid froth are triturated with 2 portions each of 25 parts by volume of benzene and filtered each time. The benzene insoluble material is saved for further treatment. The benzene soluble fraction is poured on to a column of 40 parts by weight of activated alumina (Woelm, Activity Grade I) which is then eluted first with 3 portions each of 50 parts by volume of benzene and then with 6 portions each of 50 parts by volume of benzene-acetone (9:1), the first of which benzene-acetone portions had been used for extraction of the above mentioned bezene insoluble material. The second of the 6 benzene-acetone elution fractions on removal of the solvents gives a light tan solid froth which on crystallization from methanol gives colorless prismatic needles of slightly impure deserpidine. Rechromatographing of 1 part by weight of this substance on 20 parts by weight of activated alumina (Woelm, Activity Grade I) using benzene and benzene containing 0.1 per cent methanol as eluting agents following by crystallization from methanol gives color-

less prismatic needles of pure deserpidine, melting at 228—232° C.

Deserpidine obtained according to this example can be made up into pharmaceutical preparations. For example, the following compositions in tablet or injectable form may be mentioned:—

1.	Deserpidine	0.1 mg.
2.	Lactose	53.4 mg.
3.	Gelatine	1.0 mg.
4.	Starch	40.0 mg.
5.	Magnesium stearate	0.3 mg.
6.	Talcum	5.2 mg.
		100.0 mg.

1.	Deserpidine	1.0 mg.
2.	Ethanol	0.25 cc.
3.	Propylene glycol	0.25 cc.
4.	Water	0.5 cc.
		1.0 cc.

In making the tablet a homogeneous mixture is prepared from 1 and 2, a paste is made with 3 and part of 4. The paste is mixed with 1 and 2 and the remainder of 4 to form a moist homogeneous mass which is then granulated and dried. After this, 5 and 6 are added and the product tableted.

EXAMPLE 2.

500 parts by weight of dried, finely ground roots of *Rauwolfia canescens* are extracted batch-wise with methanol at its boiling point, using the following volumes and times, and filtering each extract while hot; 2000 parts by volume, 1 hour; 1000 parts by volume, 45 minutes; 1000 parts by volume, 30 minutes; 1000 parts by volume, 30 minutes. The extracts are combined and evaporated *in vacuo* to 75 parts by volume of thick syrupy solution. After the addition of 75 parts by volume of methanol and 150 parts by volume of acetic acid of 15 per cent strength with adequate mixing, the solution is extracted with 2 portions each of 100 parts by volume of hexane. The combined hexane extracts are extracted with 15 parts by volume of acetic acid of 15 per cent strength. The latter extract is added to the above acetic acid phase which is then extracted with 3 portions each of 75 parts by volume and 1 portion of 50 parts by volume of ethylene chloride. The first 3 extracts are combined and washed with 60 parts by volume of 2N sodium carbonate solution and then with 60 parts by volume of distilled water. These washing solutions are saved and used for the washing of the 4th and final ethylene chloride extract. The combined ethylene chloride extracts are dried over sodium sulfate, filtered and evaporated *in vacuo* to a constant weight of a tan, frothy solid. 1 part by weight of this residue is dissolved in 1.5 parts by volume of warm methanol and the solution cooled to 5° C. for 18 hours, whereby crystallization of a mixture containing principally reserpine sets in.

0.665 part by weight of the above product is dissolved in 8 parts by volume of methylene chloride, treated with 0.05 part by weight of activated charcoal which is then removed by filtration, using 2 parts by volume of methylene chloride as a wash. While the methylene chloride is distilled off it is replaced by 6 parts by volume of methanol. The distillation is continued until the methylene chloride is removed and a volume of approximately 2 parts by volume of methanol remains. After standing overnight at −5°, the crystals of impure reserpine are filtered and washed with three portions each of 0.25 part by volume of cold methanol. The mother liquor and wash from the above crystals is evaporated *in vacuo* to a tan solid residue. 0.85 part by weight of this is dissolved with warming in 2.1 parts by volume of acetone. Needles crystallize from the warm solution. After standing for 2 hours at room temperature, the crystals are filtered, washed with cold acetone, and dried *in vacuo* at 50° for several hours. 0.236 part by weight of these crystals are dissolved in boiling acetone, the solution concentrated to a volume of 1.7 parts by volume, cooled at room temperature, whereupon crystallization sets in. After standing at room temperature overnight, the crystals are filtered, washed with cold acetone, and dried *in vacuo* at 50° C. for 5 hours. 0.143 parts by weight of these crystals are dissolved in 0.5 part by volume of warm methanol. The crystals dissolve readily and from the solution there crystallize rapidly rosettes of tiny prismatic needles. After standing at room temperature overnight, the crystals are filtered and washed with cold methanol. The thus obtained deserpidine melts at 228—232°.

EXAMPLE 3.

To a solution of 0.2 part by weight deserpidine in 3 parts by volume of methanol and 0.1 part by volume of methylene chloride is added 0.2 part by volume of dilute sulfuric acid (1 part by volume sulfuric acid: 4 parts by volume water). After boiling out the methylene chloride, the solution is allowed to stand at 5° for a few hours. The salt of deserpidine with sulfuric acid crystallizes with water from this solution in white needles, which after filtering and washing with methanol melt at 266—269 (dec.).

EXAMPLE 4.

0.2 part by weight of deserpidine is slurried with 1 part by volume methanol. Methanol saturated with gaseous hydrochloric acid is added until all deserpidine is in solution. The resulting solution is evaporated to dryness. From 1 part by volume 95 per cent ethanol, the hydrochloride of deserpidine crystallizes with water as needles. The needles are filtered and washed with ethanol; they melt at 253—256° (dec.).

EXAMPLE 5.

To a solution of 0.2 part by weight of deserpidine in 3 parts by volume of methanol and 0.1 part by volume of methylene chloride is added 0.05 part by volume of dilute nitric acid (1 part nitric acid: 4 parts water). Crystallization begins immediately. After cooling at 5°, the plates are filtered and washed with methanol. The thus obtained salt of deserpidine with nitric acid melts at 254—260° (dec.).

EXAMPLE 6.

0.2 part by weight of deserpidine is dissolved in 3 parts by volume of methanol and 0.1 part by volume of methylene chloride. 0.3 part by volume of oxalic acid solution (1 part by weight anhydrous oxalic acid: 10 parts by volume water) is added. After boiling out the methylene chloride, the solution is cooled at 5° for several hours. The white crystals formed are filtered and washed with methanol. The thus obtained salt of deserpidine with oxalic acid melts at 239—243° (dec.).

EXAMPLE 7.

Deserpidine may be made up, in addition to the pharmaceutical preparations described in Example 1, for example into the following preparations:—

1. Deserpidine	0.50 g.
2. Tragacanth BC	3.00 g.
3. Lactose	134.50 g.
4. Corn starch	3.75 g.
5. Talcum	7.50 g.
6. Magnesium stearate	0.75 g.
	150.00 g.

The deserpidine and tragacanth are mixed together and then mixed with the lactose. The resulting mixture is granulated with ethanol 50 per cent and passed through a No. 10 screen. It is then dried thoroughly and passed through a No. 16 screen. The granulation is then mixed with the talcum, corn starch and magnesium stearate and the resulting granulation after rescreening tableted into tablets of 150 mg. weight each.

1. Deserpidine	0.125 g.
2. Citric acid, anhydrous	0.125 g.
3. Benzyl alcohol	1.000 ml.
4. Polyethylene glycol 300	5.000 ml.
5. Water for injection to make	50.000 ml. of solution

This preparation for injection is obtained by dissolving the deserpidine in the benzyl alcohol, and adding the solution of the citric acid in 1 ml. of water for injection. After mixing the polyethylene glycol is added and the whole mixed well. Water for injection is slowly added to make up a 50 ml. solution, which is then filtered through a coarse porosity sintered glass funnel.

Furthermore, the following pharmaceutical preparation may be mentioned:—

1. Deserpidine	0.025 g.
2. Diethylaminoethyl α-cyclohexyl-α-phenyl-α-hydroxy acetate methobromide	5.000 g.
3. Tragacanth	1.125 g.
4. Corn starch	2.250 g.
5. Lactose	36.150 g.
6. Magnesium stearate	0.450 g.
	45.000 g.

This preparation is made up into 50 mg. tablets coated with shellac, sucrose, flour and titanium dioxide.

1. Deserpidine	0.100 g.
2. 1-hydrazino-phthalazine hydrochloride	25.000 g.
3. Tragacanth	4.500 g.
4. Lactose	96.025 g.
5. Corn starch	3.750 g.
6. Talcum	4.500 g.
7. Magnesium stearate	1.125 g.
8. Citric acid anhydrous	15.000 g.
	150.000 g.

This preparation is made up into 150 mg. tablets.

1. Deserpidine	0.200 g.
2. 1-hydrazino-phthalazine hydrochloride	50.000 g.
3. Tragacanth	6.000 g.
4. Lactose	132.300 g.
5. Corn starch	5.000 g.
6. Talcum	5.000 g.
7. Magnesium stearate	1.500 g.
	200.000 g.

This preparation is made up into 200 mg. tablets.

EXAMPLE 8.

1000 parts by weight of ground root of *Rauwolfia indecora* are refluxed for one hour with 4000 parts by volume of methanol. The extract is then filtered while hot. The roots are then re-extracted three times with refluxing methanol, filtering the extract hot each time, using 2000 parts by volume of methanol for one half hour, then 2000 parts by volume of methanol for one-quarter hour, then 1000 parts by volume of methanol for one-quarter hour. The combined filtrates are concentrated to 150 parts by volume. To this viscous solution 150 parts by volume of methanol and 300 parts by volume of 15 per cent acetic acid are added, and the mixture is extracted with two portions each of 200 parts by volume of hexane. Each hexane extract is reextracted with a solution of 25 parts by volume of methanol and 25 parts by volume of 15 per cent acetic acid. The combined aqueous phases and washes are then extracted four times with 200 parts by volume of benzene. These combined benzene extracts are washed with 70 parts by

volume of 20 per cent potassium carbonate, then three times with 20 per cent sodium chloride, dried over anhydrous potassium carbonate, and concentrated *in vacuo* to give a brown frothy residue. Crystallization of this material from methanol gives a first crop, m.p. 267—274° C., and a second crop, m.p. 260—270° C. The second crop is recrystallized from methanol, and the recrystallization mother liquor material crystallized and recrystallized from acetone to give deserpidine.

EXAMPLE 9.

To 500 parts by weight of ground root of *Rauwolfia canescens*, 600 parts by volume of water and 1500 parts by volume of thiophene-free benzene are added and the mixture is refluxed for one hour, then filtered while hot. The root is then re-extracted twice by refluxing with 1000 parts by volume of benzene and 100 parts by volume of water, once for 30 minutes and once for 15 minutes, and the extracts filtered while hot each time. These filtrates are combined and concentrated *in vacuo* to give a tan solid. This is taken up in 8.0 parts by volume of methanol and 4.0 parts by volume of hexane. A small amount of insoluble material which settles out overnight is filtered. The filtrate is concentrated to dryness *in vacuo* and taken up in 10 parts by volume of methanol. Enough 17 per cent nitric acid is added slowly and with shaking to bring the *p*H to 3. The solution is then diluted with 2 parts by volume of ether and allowed to stand at room temperature for 20 hours, during which a crystalline nitrate settles out. This material, m.p. 252—256° C. (dec.), is filtered, dissolved in methanol, and concentrated aqueous ammonia added till the solution is slightly basic. The crystalline base, m.p. 252—256° C., which settles out is filtered and recrystallized from methanol to give crystals, m.p. 262—265° C. The mother liquor material from this crystallization is taken down to dryness, then dissolved in a little acetone to give deserpidine in needle-like crystals.

EXAMPLE 10.

5000 parts by weight of dried and finely ground roots of *Rauwolfia canescens* are extracted batch-wise with methanol at its boiling point using the following volumes and times and filtering each extract while hot: 11000 parts by volume, 2 hours; 5000 parts by volume, 1 hour; 6000 parts by volume, 1 hour; 6000 parts by volume, 1 hour. The combined extracts are evaporated *in vacuo* to a volume of 4000 parts by volume. Insoluble solids are removed by filtration and the filtrate is further concentrated under vacuum to a volume of 900 parts by volume of a red-brown viscous syrup. 4000 parts by volume of water are added and then concentrated aqueous ammonia (ca. 15 parts by volume) until the solution has a *p*H of 7.2. After standing overnight at room temperature, the supernatant is decanted from a red-brown tarry residue, which is dried under vacuum and at room temperature for 3 days. A partially dried and very hydroscopic dark brown solid is obtained.

13.6 parts by weight of the above solid is dissolved in 80 parts by volume of methanol and 80 parts by volume of 1.7 per cent phosphoric acid. This solution is extracted twice with 50 parts by volume of hexane. The hexane extracts are washed with a mixture of 10 parts by volume of methanol and 10 parts by volume of 1.7 per cent phosphoric acid. These washes plus the original aqueous acidic phase are then extracted four times with methylene chloride, twice with 50 parts by volume and twice with 20 parts by volume. The fourth extract is kept separate and used subsequently as a wash of the sodium carbonate and sodium chloride washes. The first three extracts are combined and washed with 50 parts by volume of 5 per cent sodium carbonate, then twice with 20 parts by volume of 20 per cent sodium chloride solution. The combined extracts are then dried over anhydrous potassium carbonate and concentrated *in vacuo* to give a brown solid. This gives on crystallization from methanol a crystalline material, m.p. 263—266° C., which is recrystallized. The re-crystallization mother liquor is taken down to dryness and taken up in a little acetone, whereupon deserpidine crystallizes in fine needles. Deserpidine can also be crystallized from ethyl acetate; it melts then at 228—232° C.

EXAMPLE 11.

To 500 parts by weight of ground root of *Rauwolfia canescens*, 600 parts by volume of water and 1500 parts by volume of ethylene chloride are added, and the mixture refluxed for 55 minutes, then filtered while still hot. The root is then re-extracted twice by refluxing with 900 parts by volume ethylene chloride and 100 parts by volume of water for twenty minutes, and filtering the extract hot each time. These filtrates are combined and concentrated *in vacuo* to give a tan solid.

This material is triturated with warm hexane three times, twice with 50 parts by volume and once with 15 parts by volume, filtering each time. The hexane insoluble material is taken up in 10 parts by volume methanol, and 17 per cent nitric acid added slowly and with shaking till a *p*H of about 3 is reached. 2 parts by volume of ether are then added and the solution allowed to stand at room temperature for 3 days, then at 5° C. for one day, after which the nitrate formed is filtered off. From the mother liquor two more crops of nitrate are obtained, the first after one day at room temperature, and the second after eight days at room temperature. These three crops are combined and crystallized twice from methanol to give a nitrate, m.p. 234—242° C. (dec.). This is dissolved in approximately 5 parts by volume of methanol and a few drops concen-

trated ammonium hydroxide added. After a few hours a basic material, m.p. 248—252° C. separates and is filtered. This is recrystallized from 5 parts by volume methanol to give crystals of melting point 257—266° C. The mother liquor of this material is concentrated to dryness and taken up in acetone to give crystalline deserpidine.

EXAMPLE 12.

5000 parts by weight of dried and finely ground roots of *Rauwolfia canescens* are extracted batch-wise with methanol at its boiling point using the following volumes and times and filtering each extract while hot: 11000 parts by volume, 2 hours; 5000 parts by volume, 1 hour; 6000 parts by volume, 1 hour; 6000 parts by volume, 1 hour. The combined extracts are evaporated *in vacuo* to a volume of 4000 parts by volume. Insoluble solids are removed by filtration, and the filtrate is further concentrated under vacuum to a volume of 900 parts by volume of a red-brown viscous syrup. 4000 parts by volume of water are added and then concentrated aqueous ammonia (ca. 15 parts by volume) until the solution has a *p*H of 7.2. After standing overnight at room temperature, the supernatant is decanted from a red-brown tarry residue, which is dried under vacuum and at room temperature for 3 days. A partially dried and very hygroscopic dark brown solid is obtained.

20 parts by weight of this material is subjected to electrodialysis, employing a 4 compartment cell, the compartments of which are separated by suitable membranes, for example, cellophane membranes. The word "Cellophane" is a Registered Trade Mark. Compartment I consists of a platinum cathode and 250 parts by volume of water through which carbon dioxide is bubbled. Compartment II contains 20 parts by weight of the above crude alkaloid extract dissolved in 100 parts by volume of acetic acid and 35 parts by volume of methanol. Compartment III contains 150 parts by volume of water and Compartment IV, 100 parts by volume of 50 per cent sulfuric acid and a platinum anode. The cell is operated for 55 hours at an average current of 200 milliamperes and an initial voltage of 310 volts which drops to an average of 30 volts within 2 hours. The average temperature of the cell is 30° C. Twice during the operation of the cell the light amber-colored solution is withdrawn from Compartment I and replaced with fresh water. The three portions of solution from Compartment I are combined and kept under refrigeration until worked up.

To these solutions from Compartment I, totalling approximately 350 parts by volume, concentrated ammonium hydroxide is added slowly and with shaking until the solution is at *p*H 7.5—8. A total of 24 parts by volume is used. This produces a tan precipitate which is filtered after standing at 5° C. for one hour. After drying this material, it is triturated with two portions each of 50 parts by volume of warm acetone containing 2 per cent methanol, then once with 50 parts by volume of acetone containing 5 per cent methanol. The insoluble material is filtered and the filtrate shaken with 2.0 parts by weight of alumina and re-filtered through Hyflo Filtercel (Registered Trade Marks). Concentrating the filtrate to dryness yields a tan solid material. This is taken up in 5 parts by volume of methanol. After addition of 0.5 part by volume of water and drop concentrated ammonium hydroxide, and standing at 5° C. for 18 hours, a crystalline product, m.p. 262—265° C. is obtained. A second crop, m.p. 260—264° C. is obtained after 6 days at 5° C. These two crops of crystals are combined and re-crystallized from methanol. The mother liquor, on concentrating to dryness and crystallizing from acetone, gives crystalline deserpidine.

EXAMPLE 13.

500 parts by weight of dried and finely ground roots of *Rauwolfia canescens* are covered with 2000 parts by volume of 15 per cent acetic acid and allowed to stand at room temperature for 18 hours. The extract is then filtered and the root material is stirred for one hour with 2000 parts by volume of 15 per cent acetic acid. After filtration, the root material is stirred for one-half hour with 1000 parts by volume of 15 per cent acetic acid and filtered. The three extracts are combined and extracted with 3 portions each of 500 parts by volume of benzene. The combined benzene extracts are washed with 260 parts by volume of a 23 per cent potassium carbonate solution. At this point the *p*H of the extract is 9. The benzene extract is made neutral by washing with 300 parts by volume of water. After drying over sodium sulfate and evaporating the benzene, a light tan froth is obtained. 0.64 part by weight of this is dissolved in 1 part by volume of methanol. White crystals, melting at 258—262° C. are obtained. 0.31 part by weight of this crystalline material is dissolved in methylene chloride and filtered. Methanol is added and the methylene chloride boiled out. A first and second crop, melting at 259—263° C. and 249—255° C. respectively, are taken. The mother liquor of the second crop is evaporated to dryness and the residue is crystallized from a small volume of acetone. This gives deserpidine as white needles.

EXAMPLE 14.

5000 parts by weight of dried and finely ground roots of *Rauwolfia canescens* are extracted batch-wise with methanol at its boiling point using the following volumes and times and filtering each extract while hot: 11000 parts by volume, 2 hours; 5000 parts by volume, 1 hour; 6000 parts by volume, 1 hour; 6000 parts by volume, 1 hour. The combined extracts are evaporated *in vacuo* to a

volume of 4000 parts by volume. Insoluble solids are removed by filtration, and the filtrate is further concentrated under vacuum to a volume of 900 parts by volume of a red-brown viscous syrup. 4000 parts by volume of water are added and then concentrated aqueous ammonia (ca. 15 parts by volume) until the solution has a *p*H of 7.2. After standing overnight at room temperature, the supernatant is decanted from a red-brown tarry residue, which is dried under vacuum and at room temperature for 3 days. 170 parts by weight of a partially dried and very hygroscopic dark brown solid is obtained.

40 parts by weight of the above solid are purified by triturating with 200 parts by volume of 95 per cent ethanol, heating to boiling and filtering. The insoluble portion is discarded and the filtrate is evaporated to dryness. The brown solid residue is subjected to a distribution between equal volumes of the upper and lower phases from an equilibration of chloroform with an equal volume of a 1:1 mixture of methanol and water. The distribution is carried out over 6 separatory funnels, each containing 200 parts by volume of the upper phase and 200 parts by volume of the lower phase. The lower phases of the 5th and 6th separatory funnels are dried over sodium sulfate, filtered, evaporated and the residues combined. 9 parts by weight of this brown solid residue is triturated with 50 parts by volume of benzene and filtered. The benzene insoluble material is triturated again with 50 parts by volume of benzene and filtered. The insoluble portion is discarded and the soluble portions are combined and chromatographed on 180 parts by weight alumina (Woelm; Activity I, neutral). Fractions of 250 parts by volume are collected starting with benzene as the first eluant; followed by benzene containing 0.1 per cent, 0.2 per cent, 0.5 per cent and 1 per cent methanol. White crystalline material, m.p. 260—266° C. is obtained from the fractions eluted with benzene containing 0.5 per cent methanol. This material is recrystallized from a small volume of methanol giving crystals, m.p. 263—268° C. which are filtered off. A second crop of small white prisms is then obtained, which represents deserpidine.

EXAMPLE 15.

To a solution of 0.3 part by weight of deserpidine in 2 parts by volume of chloroform is added 0.04 part by volume of 85 per cent phosphoric acid in 1 part by volume of methanol. The solution is evaporated to dryness *in vacuo* and the resulting pale-yellow solid is dried over phosphorus pentoxide for three days under vacuum and at room temperature. The dried solid is dissolved in 2 parts by volume methanol. Addition of 20 parts by volume ether gives a white precipitate which is filtered, washed with ether, and dried over phosphorus pentoxide for 18 hours under vacuum and at room temperature. The white powder sinters at 185° and decomposes at about 220° C. The thus obtained phosphoric acid salt of deserpidine contains about 3 moles of water.

WHAT WE CLAIM IS:—

1. Process for the manufacture of a pure active substance, consisting in isolating deserpidine which melts at about 228—232° C. and possesses an approximate optical rotation of $[\alpha]_D^{24} - 137 \pm 1°$ (in absolute chloroform) from the mother liquor from a reserpine crystallisation obtainable in a process for the isolation of reserpine from plants of the *Rauwolfia* species, the isolation being carried out with the use of at least one of the following methods:—

(a) Treating the mother liquors with acids or salts suitable for salt formation with weak bases and separating the salts obtained;

(b) Decomposing the mother liquors; and

(c) Crystallisation;

and, if desired, preparing the salts thereof.

2. Process according to Claim 1, consisting in extracting plant material from plants of the *Rauwolfia* species or crude extracts therefrom with an organic solvent only partially miscible with water, separating reserpine and isolating deserpidine from the extracts obtained.

3. Process according to Claim 1 or 2, consisting in using root material from *Rauwolfia* plants as starting material.

4. Process according to any one of Claims 1—3, consisting in using *Rauwolfia canescens* as starting material.

5. Process according to any one of Claims 1—3, consisting in using *Rauwolfia vomitoria Afz.* as starting material.

6. Process according to any one of Claims 1—3, consisting in using *Rauwolfia hirsuta* as starting material.

7. Process according to any one of Claims 1—3, consisting in using *Rauwolfia tetraphylla* as starting material.

8. Process according to any one of Claims 1—3, consisting in using *Rauwolfia indecora* as starting material.

9. Process according to any one of Claims 1—3, consisting in using *Rauwolfia cubana* as starting material.

10. Process according to any one of Claims 2—9, consisting in using extracts obtained with lower alcohols as starting material.

11. Process according to any one of Claims 2—10, consisting in using extracts obtained with an aqueous acid agent as starting material.

12. Process according to any one of Claims 2—11, consisting in using crude extracts treated with a lipoid solvent as starting material.

13. Process according to any one of Claims 2—12, consisting in carrying out the extrac-

tion with an organic solvent only partially miscible with water in the presence of a polar solvent.

14. Process according to any one of Claims 2—13, consisting in carrying out the extraction with an organic solvent only partially miscible with water in the presence of a polar solvent and water.

15. Process according to any one of Claims 13—14 consisting in using a lower aliphatic alcohol as polar solvent.

16. Process according to any one of Claims 2—15, consisting in using a halogenated aliphatic hydrocarbon as organic solvent only partially miscible with water.

17. Process according to any one of Claims 2—16 consisting in treating a crude extract obtainable with a lower alcohol with an aqueous acid agent for the preparation of a sedative extract, treating the latter first with a lipoid solvent, then extracting with an organic solvent only partially miscible with water, separating reserpine and isolating deserpidine from the extract obtained.

18. Process according to any one of Claims 11 and 17, consisting in using an aqueous solution of a lower fatty acid as aqueous acid agent.

19. Process according to any one of Claims, 11, 17 and 18 consisting in using dilute acetic acid as aqueous acid agent.

20. Process according to any one of Claims 1—19, consisting in treating crude extracts obtained from plants of the *Rauwolfia* species with acids or salts which are suitable for forming salts with weak bases, separating reserpine and isolating deserpidine from the salts obtained.

21. Process according to any one of Claims 1—20, consisting in isolating deserpidine with the use of an adsorption agent and by subsequent crystallization.

22. Process according to any one of Claims 1—21, consisting in isolating deserpidine by chromatography, especially over aluminium oxide and subsequent crystallization.

23. Process according to any one of Claims 1—22, consisting in crystallising deserpidine with the use of an alcohol.

24. Process according to any one of Claims 1—23, consisting in crystallising deserpidine with the use of methanol.

25. Process according to any one of Claims 1—24, consisting in carrying out the treatment with an adsorption agent or the crystallization after removing reserpine by crystallization from methanol.

26. Process for the manufacture of deserpidine, conducted substantially as described in any one of the examples herein.

27. Deserpidine, melting at about 228—232° C. and having an optical rotation $[\alpha]_D^{24} = -137 \pm 1$ (in chloroform).

28. A salt of deserpidine.

29. The salt of deserpidine with hydrochloric acid.

30. The salt of deserpidine with nitric acid.

31. The salt of deserpidine with sulfuric acid.

32. The salt of deserpidine with oxalic acid.

33. The salt of deserpidine with phosphoric acid.

34. A pharmaceutical composition comprising deserpidine substantially free from combined alkaloids and other materials associated with plants of the *Rauwolfia* species, or salts thereof, and a pharmaceutical carrier.

35. A pharmaceutical composition comprising deserpidine substantially free from combined alkaloids and other materials associated with plants of the *Rauwolfia* species, or salts thereof, and a solid pharmaceutical carrier.

36. A pharmaceutical composition comprising deserpidine substantially free from combined alkaloids and other materials associated with plants of the *Rauwolfia* species, or salts thereof, and a liquid pharmaceutical carrier.

37. A pharmaceutical composition comprising deserpidine substantially free from combined alkaloids and other materials associated with plants of the *Rauwolfia* species, or salts thereof, and a pharmaceutical carrier in dosage unit form.

38. A pharmaceutical composition comprising deserpidine substantially free from combined alkaloids and other materials associated with plants of the *Rauwolfia* species, or salts thereof, and a solid pharmaceutical carrier in tablet dosage unit form.

39. A pharmaceutical composition comprising deserpidine substantially free from combined alkaloids and other materials associated with plants of the *Rauwolfia* species, or salts thereof, and a solid pharmaceutical carrier in tablet dosage unit form containing 0.05—100 mg. of active substance.

40. A pharmaceutical composition comprising deserpidine substantially free from combined alkaloids and other materials associated with plants of the *Rauwolfia* species, or salts thereof, and a solid pharmaceutical carrier in tablet dosage unit form containing 0.1—20 mg.

41. A pharmaceutical composition comprising deserpidine substantially free from combined alkaloids and other materials associated with plants of the *Rauwolfia* species, or salts thereof, and a liquid pharmaceutical carrier in ampouled dosage unit form.

42. A pharmaceutical composition comprising deserpidine substantially free from combined alkaloids and other materials associated with plants of the *Rauwolfia* species, or salts thereof, and a liquid pharmaceutical carrier in ampouled dosage unit form containing 0.05—100 mg. of active substance.

43. A pharmaceutical composition comprising deserpidine substantially free from combined alkaloids and other materials associated with plants of the *Rauwolfia* species, or salts thereof, and a liquid pharmaceutical carrier in

ampouled dosage unit form, containing 0.1—20 mg. of active substance.

ABEL & IMRAY,
Agents for the Applicants,
Quality House, Quality Court, Chancery Lane,
London, W.C.2.

Leamington Spa: Printed for Her Majesty's Stationery Office, by the Courier Press.—1959.
Published at The Patent Office, 25, Southampton Buildings, London, W.C.2, from which copies may be obtained.